Immunohistochemical Localization of Carcinoembryonic Antigen in Adenocarcinoma of the Endocervix and the Endometrium

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Immunohistochemical Localization of Carcinoembryonic Antigen in Adenocarcinoma of the Endocervix and the Endometrium. Tohoku J. exp. Med., 1985, **146** (1), 27-32 — Immunoperoxidase localization of carcinoembryonic antigen (CEA) was performed on tissue sections of adenocarcinoma of endocervical and endometrial origin to clarify the criteria for their differentiation. Twenty-two (96%) of 23 endocervical and 32 (70%) of 46 endometrial adenocarcinomas revealed positive CEA immunostain. The extent of staining did not seem to correlate with the degree of differentiation in either kind of cancer. Among the positive cases, CEA staining was located over whole cytoplasm in 18 (80%) of 22 positive endocervical adenocarcinomas and on apical surface in 24 (75%) of 32 positive endometrial adenocarcinomas. The pattern of CEA distribution in endocervical adenocarcinoma of endometrioid type was not similar to that observed for primary endometrial adenocarcinoma. ——— immunohistochemistry; carcinoembryonic antigen; endocervical adenocarcinoma; endometrial adenocarcinoma

Carcinoembryonic antigen (CEA) first described by Gold and Freedman (1965) has now become one of the best known tumor markers. Although CEA was originally described as a tumor associated antigen of the colon, it is demonstrated in serum of patients with various kinds of neoplasms.

In patients with gynecological malignancies, CEA is demonstrated in serum by radioimmunoassay and in tissue by immunohistochemistry. Wahlström and colleagues (1979) suggested that the possibility of presence of CEA is much higher in an adenocarcinoma of endocervical origin as compared with that of endometrial origin and that this might be an important consideration in making differential diagnosis. On the other hand, CEA has also been detected in some cases of endometrial adenocarcinoma by several other authors (Goldenberg et al. 1978; Cohen et al. 1982; Ueda et al. 1983; Okudaira et al. 1984).

The aim of the present study is to clarify the difference in localization of CEA

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between endocervical and endometrial adenocarcinomas and to distinguish these two types of uterine cancer.

MATERIALS AND METHODS

The materials consisted of 23 cases of endocervical and 46 cases of endometrial adenocarcinomas. All cases were collected from the specimens obtained by hysterectomy performed at the Department of Obstetrics and Gynecology, Tohoku University Hospital and National Sendai Hospital (kindly provided by Dr. Tsuneo Namiki). The primary site of carcinoma was clinically and histologically defined in all cases.

All slices were recut from routine formalin-fixed and paraffin-embedded blocks; these materials were obtained during the period from 1981 to 1984. Three micron serial sections were deparaffinized and stained with hematoxylin-eosin and alcian blue at pH 2.5.

Immunoperoxidase for CEA. Immunohistochemical study was performed by the ABC method (Hsu et al. 1981) using Vectastain rabbit IgG kit (Vector Laboratories). Anti-CEA rabbit antiserum (Dakopatts) was absorbed with perchloric acid extract of normal human spleen tissue for 12 hr at 4°C before use to remove reactivity with non-specific cross-reacting antigen (Wagener et al. 1978). Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in anhydrous methanol. Tissue sections were then rinsed and incubated for 20 min at 37°C in 0.1% trypsin 1:250 (Difco) in 0.05 M Tris-HCl buffer pH 7.6 with the addition of 0.1% CaCl₂ (Hautzer et al. 1980). The sections were rinsed with phosphate-buffered saline (PBS), and then treated sequentially with normal goat serum (1:20, 15 min), anti-CEA (1:200, 30 min), biotinylated anti-rabbit IgG (1:200, 30 min) and Avidin-Biotin peroxidase Complex (1:100, 30 min). Each reaction was carried at room temperature in a moist chamber, with intervening cold PBS washes. The bound peroxidase was visualized by immersing the slices for 3–4 min at room temperature in a solution of 0.02% diaminobenzidine tetrahydrochloride (Dotite) and 0.005% hydrogen peroxide containing 10 mM sodium azide. The nuclei were counterstained with methyl green and the slices were dehydrated and mounted. The primary antiserum was replaced with normal rabbit serum 1:20 as a negative control.

Evaluation of the staining. If any small portion of the cancer tissue showed a positive staining, the tissue was labelled as weakly positive or “plus”, whereas if more than 50% of the cancer cells showed a positive staining, the sample was labelled as strongly positive or “plus plus”.

RESULTS

The results of CEA localization of 23 cases of endocervical and 46 cases of endometrial adenocarcinomas are summarized in Table 1 and illustrated in Figs. 1, 2, 3, 4 and 5. Twenty-two cases (96%) of 23 endocervical adenocarcinomas

TABLE 1. *CEA staining in adenocarcinoma of the uterus*

	(-)	CEA (+)	(#)
Endocervical adenocarcinoma	1	11	11
Endometrial adenocarcinoma	14	31	1

Fig. 1 (left). Endocervical adenocarcinoma with diffuse positive staining for CEA over whole cytoplasm ($\times 100$).

Fig. 2 (right). Endocervical adenocarcinoma with positive staining for CEA on apical surface and luminal secretion ($\times 100$).

Fig. 3 (left). Endometrial adenocarcinoma showing positive staining for CEA on apical surface ($\times 90$).

Fig. 4 (middle). Endometrial adenosquamous carcinoma showing positive staining for CEA on cytoplasm of the squamous portion ($\times 90$).

Fig. 5 (right). Endometrial adenocarcinoma showing diffuse positive staining for CEA over whole cytoplasm ($\times 90$).

TABLE 2. *Degree of differentiation and CEA immunostain in adenocarcinoma of the uterus*

	Endocervical adenocarcinoma (N=23)			Endometrial adenocarcinoma (N=46)		
	(-)	CEA (+)	(#)	(-)	CEA (+)	(#)
G1	1	6	8	10	21	0
G2	0	5	1	2	7	1
G3	0	0	2	2	3	0

G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

TABLE 3. *Distribution of positive CEA immunostain in adenocarcinoma of the uterus*

	Whole cytoplasm	Apical surface	Squamous portion
Endocervical adenocarcinoma	18	4	0
Endocervical type	11	2	
Endometrioid type	6	2	
Endometrial adenocarcinoma	8	24	7

revealed positive CEA immunostain. One negative case was clear cell adenocarcinoma. Among 22 positive cases, 11 were weakly positive and other 11 were strongly positive.

Thirty-two cases (70%) of 46 endometrial adenocarcinomas showed positive CEA immunostain. Among the positive cases, 31 were weakly positive with only one case labelled as strongly positive.

Table 2 shows the degree of tumor differentiation and the extent of CEA staining. No correlation was noted for either endocervical or endometrial adenocarcinomas. Moreover, comparison of CEA staining with alcian blue staining revealed no correlation for either type of uterine cancer.

Table 3 shows the distribution of positive CEA immunostain in endocervical and endometrial adenocarcinomas. CEA was present on whole cytoplasm in 18 cases (80%) of 22 positive endocervical adenocarcinomas (Fig. 1). In 4 cases, CEA was focally stained on apical surface (Fig. 2). In two cases of cervical adenocarcinoma with squamous component, CEA was negative in the squamous portion.

In contrast, CEA was focally stained on apical surface or apical surface plus squamous portion in 24 cases (75%) of 32 positive endometrial adenocarcinomas (Figs. 3 and 4). Only 8 cases showed positive CEA staining over whole cyto-

plasm of tumor cells (Fig. 5).

Both endocervical and endometrioid type of endocervical adenocarcinomas revealed positive CEA staining over whole cytoplasm more frequently than on apical surface. That is to say, the pattern of CEA distribution in endometrioid type of endocervical adenocarcinoma was not similar to that in primary endometrial adenocarcinoma.

After all, CEA was immunohistochemically detected on whole cytoplasm in most of endocervical adenocarcinomas and on apical surface in most of endometrial adenocarcinomas. This result was statistically significant (chi-square test, $p < 0.001$).

DISCUSSION

It is sometimes difficult to establish differential diagnosis on endocervical and endometrial adenocarcinomas in routine histological specimens. Several authors reported that these two types of uterine adenocarcinomas could be differentiated by immunoperoxidase technique.

Wahlström et al. (1979) suggested that 80% of endocervical adenocarcinomas show positive CEA immunostain, while all endometrial adenocarcinomas but adenosquamous carcinomas are CEA negative. In several other papers, positive rate for CEA by immunoperoxidase in cervical adenocarcinoma was reported to be 92–100% (Cohen et al. 1982; Ueda et al. 1983; Speers et al. 1983), while it varies from 8 to 52% in endometrial adenocarcinoma (Goldenberg et al. 1978; Cohen et al. 1982; Ueda et al. 1983; Okudaira et al. 1984).

In the present study, 96% of endocervical adenocarcinomas showed positive CEA staining in agreement with the results reported in other series. Wahlström et al. (1979) reported that all of their 11 cases of endocervical clear cell adenocarcinoma were CEA negative. In our series, the only one case that did not show CEA staining in endocervical adenocarcinomas was clear cell adenocarcinoma. These results suggest that clear cell and pure endocervical adenocarcinoma may have different origin.

On the other hand, positive rate for CEA immunostain in endometrial adenocarcinomas in our series was considerably higher than in the previous reports. In addition, the distribution of positive CEA immunostain was quite different between endocervical and endometrial adenocarcinomas in our study. CEA was stained over whole cytoplasm in most of endocervical adenocarcinomas and on apical surface in most of endometrial adenocarcinomas, respectively. We used ABC method, which is more sensitive than the conventional immunoperoxidase technique such as PAP method. So, it is supposed that this sensitive method visualized the trace amount of CEA deposited on apical surface, raising the positive rate for CEA staining in endometrial adenocarcinomas. Therefore, the difference of CEA distribution rather than simple CEA positivity might be more useful to distinguish endocervical adenocarcinoma from endometrial one.

Endocervical adenocarcinoma of endometrioid type is said to develop from ectopic endometrial glands within the cervix (Noda et al. 1983). But in this study, the endocervical adenocarcinoma of endometrioid type and the primary endometrial adenocarcinoma showed different distribution in CEA staining. From these results, it is supposed that their functional characteristics are different.

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